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Depth-dependent response of soil aggregates and soil organic carbon content to long-term elevated CO_2 in a temperate grassland soil

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ABSTRACT

Facing rising atmospheric CO_2 concentrations, subsoils may play an important role in the global carbon (C) cycle due to the presence of unsaturated mineral surfaces. Further, macroaggregation is considered a crucial process influencing C sequestration. However, analyses on subsoil aggregation and C retention processes under long-term elevated CO_2 (eCO_2) are lacking. In this study we investigated the long-term effect of +20% above ambient CO_2 concentration (corresponds to conditions reached 2035–2045) in a temperate grassland ecosystem at the Giessen Free Air CO_2 Enrichment (Gi-FACE), Germany. A depth-dependent response of macroaggregation to eCO_2 was observed: While in subsoil (15–45 cm depth) macroaggregation increased under eCO_2, no CO_2 induced change in macroaggregation was detected in topsoil (0–15 cm). Increased macroaggregation in subsoil coincided with higher SOC content of large macroaggregates (LM). Mean residence time (MRT) of SOC in aggregate-size classes were not different among each other under eCO_2. However, macroaggregates and bulk soil differed in their MRT between soil depths. Despite increased macroaggregation and an estimated high SOC sequestration potential in subsoil we could not observe an increase in SOC content of bulk soil.

1. Introduction

Since soil organic carbon (SOC) presents the largest terrestrial pool of C (Amundson, 2001), its potential to store additional C from the atmosphere has been widely discussed in the scientific literature (Stockmann et al., 2013). Accordingly, the 4 per mille initiative considers SOC sequestration as a contribution to mitigate climate change (Minasny et al., 2017) and calls out for accounting the rate of SOC sequestration and to identify mechanisms increasing SOC stocks.

It is widely accepted that SOC sequestration depends on the distribution of soil organic matter (SOM) in soil aggregates. The potential to physically protect certain SOM fractions from decomposition varies with aggregate-size class, which governs their residence time in soil (Tisdall and Oades, 1982; Van Veen and Kuikman, 1990; Jastrow et al., 1996). Further, subsoils may play an important role in the global C cycle due to their high mean residence times (MRT) relative to topsoil (Rumpel and Kögel-Knabner, 2011) and the presence of unsaturated mineral surfaces which was shown to be related to the formation of macroaggregates and C accrual (Kaiser and Guggenberger, 2003; Poirier et al., 2014).

However, in view of rising atmospheric CO_2 concentrations, it remains unclear how elevated CO_2 (eCO_2) affects the distribution of SOC to soil aggregate-size classes in different soil depths, the associated MRT and the resulting SOC content. For effective C sequestration, it is relevant that additional C is allocated to pools with long-term stabilization and not fast cycling pools.

It has been reported that eCO_2 may alter many factors known to influence the distribution of soil aggregate-size classes (Díaz, 1995; Eviner and Chapin, 2002). For example, eCO_2 can alter the vegetation community composition and related fungal biomass which was shown to affect aggregate stability (Rillig et al., 2002). Six et al. (2001) showed that eCO_2 changed the quality of residue inputs and enhanced the proportion of recently photosynthesized C with increasing aggregate size. They concluded that the quantity and quality of residues, which was altered by eCO_2, determined the turnover time of macroaggregates. Furthermore, it was reported that eCO_2 enhanced rhizodeposition which may stimulate fungal biomass (Phillips et al., 2006) that may serve as a binding-agent for macroaggregates (Tisdall and Oades, 1982).

Free-Air CO_2 Enrichment (FACE) experiments proofed to be a powerful approach to examine ecosystem responses to eCO_2 (Ainsworth and Long, 2005). FACE experiments allow the investigation of intact ecosystems which are exposed in-situ to eCO_2 concentration without enclosure. Nine FACE studies that investigated the effect of eCO_2 on the

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distribution of soil aggregate-size classes across a variety of ecosystems showed contrasting results (Table S1). Eight out of nine FACE studies reported results after short-term enrichments (< 10 years of CO_2 enrichment) which may not be representative of long-term dynamics. Not all of the studies incorporated measurements of SOC-content and some focused on microbial responses within aggregates (Dorodnikov et al., 2009; Nie et al., 2014) or the influence of arbuscular mycorrhizal fungi to aggregation changes (Rillig et al., 2001). In five of the FACE studies, assessment of aggregate-size class distribution was limited to the topsoil, while two studies analyzed pooled samples of top- and subsoil, consequently losing any depth-dependent information. As a result, only very limited information is available on how the distribution of soil aggregate-size classes responds to soil depth under long-term eCO_2.

To our knowledge only one other FACE study (Hofmockel et al., 2011) exists to date that investigated long-term effects (> 10 years) of eCO_2 on the distribution of soil aggregate-size classes and SOC-content. Hofmockel et al. (2011) demonstrated that eCO_2 changed C turnover of different particle-size classes in a forest soil suggesting a eCO_2 induced priming of older, relatively stable SOC.

Thus our main objective was to quantify long-term and depth-dependent effects of eCO_2 on the abundance of soil aggregate-size classes and soil C dynamics in a FACE-experiment which, to our knowledge, has not been investigated in detail so far. Since the Gi-FACE is located on temperate managed grassland our study complements the results from the long-term forest FACE study (Hofmockel et al., 2011).

In this study we investigated if eCO_2 (1) affected the distribution of soil aggregate-size classes at different soil depths; (2) induced a change in aggregate and bulk SOC content at different soil depths and (3) affected the mean residence time (MRT) and distribution of newly sequestered C (C_{new}) in soil aggregates and bulk soil at different depths.

Based on studies reporting higher C sequestration potential in subthan topsoil (Kaiser and Guggenberger, 2003; Poirier et al., 2014) we hypothesized that (i) topsoil will be close to C saturation and will show small increases in SOC content under long-term eCO_2 and (ii) subsoil will have a higher C saturation deficit and will therefore increase to a higher extent in SOC relative to topsoil under eCO_2.

2. Materials and methods

2.1. Study site and design

The Giessen Free Air Carbon Enrichment (Gi-FACE) experiment, is located on permanent semi-natural grassland. It is situated near Giessen, Germany (50°32′N and 8°41.3′E) at an elevation of 172 m above sea level.

The set-up and performance of the Gi-FACE system has been described in detail by Jäger et al. (2003) and Andresen et al. (2017). In brief, from May 1998 until present, atmospheric CO_2 concentrations were enriched by 20% above ambient, all-year-round during daylight hours. From May 1998 to June 2004 the $\delta^{13}C$ signature of the CO_2 used for enrichment was −25‰ (compared to ambient atmospheric CO_2 (aCO_2): −8‰). From July 2004 onwards the $\delta^{13}C$ signature of the CO_2 was changed to −48‰ without altering the CO_2 concentration. The CO_2 enrichment was applied in three rings, each eight meter in diameter (E plots). Three equally sized control plots were maintained at aCO_2 levels (A plots). The experimental design was a randomized block design. A block consisted of two plots to which ambient and eCO_2 treatments were randomly assigned. A characteristic attribute of the study site is a soil moisture gradient, resulting from a gradual terrain slope (2–3°) and varying depths of a subsoil clay layer. Within each of the three blocks, soil moisture conditions were relatively homogeneous (Jäger et al., 2003). The soil of the study site is classified as a Fluvic Gleysol (FAO classification). The soil texture and the depth of the clay layer is presented in Table 1.

The vegetation is an Arrhenatheretum elatioris Br.Bl. Filipendula ulmaria subcommunity, dominated by *Arrhenaterum elatium, Galium album* and *Geranium pratense*. At least 12 grass species, 15 non-leguminous herbs and 2 legumes are present within a single ring. For at least 100 years, the grassland has not been ploughed. Since at least 60 years, it was managed as a hay meadow with two cuts per year, and fertilized at the rate of 50–100 kg N ha^{-1} yr^{-1}. From 1996, fertilizer was applied in mid-April with granular mineral calcium-ammonium-nitrate fertilizer at the rate of 40 kg N ha^{-1} yr^{-1} (Kammann et al., 2008).

Table 1
Soil texture in the soil profile of each ring pair at the Gi-FACE study site according to Lenhart (2008).

Horizon	Lower horizon boundary	Sampling depth	Depth of clay layer	Sand	Silt	Clay	Silt and clay
	(cm)			(%)			
Ring pair 1							
Ah	10	2–7	128–155	43.25	39.00	17.75	56.75
M	32	12–17		40.89	42.13	16.97	59.10
SwM	78	40–45		48.10	51.90	nd	51.90
Ring pair 2							
Ah	12	2–7	48–110	59.26	20.89	19.85	40.74
MSw	42	15–20		34.52	40.50	24.98	65.48
GoSw	65	50–55		35.34	52.33	12.33	64.66
Ring pair 3							
Ah	12	2–7	65–135	9.98	58.13	31.89	90.02
M	20	15–20		9.78	55.56	34.66	90.22
MSw	50	40–45		14.94	50.56	34.50	85.06

nd: not determined.

2.2. Soil sampling

Soil samples were taken at nine sampling dates (April 1998, June 2004, December 2004, July 2005, December 2005, June 2006, June 2007, November 2011 and December 2015) in 0–7.5 cm depth. After six (June 2004), nine (June 2007) and 13 years (November 2011) of CO_2 enrichment soil samples were taken in 0–7.5, 7.5–15, 15–30 and 30–45 cm depth (soil sampler: Ejkelkamp, Giesbeek, The Netherlands) with three sub-samples per plot in each depth. Soils were passed through an 8 mm sieve and air-dried. Subsequently, roots were picked out with tweezers until all visible roots were removed. The soil samples were split partly for bulk soil analysis and 80 g of the samples were used for the wet sieving procedure to separate soil aggregate-size classes.

2.3. Soil aggregate fractionation

Soil samples were separated into four aggregate-size classes by wet sieving of 80 g of soil according to a method adapted from Cambardella and Elliott (1993). Soil samples were submerged for 2 min in deionized water on top of the 2000 µm sieve and subsequently a series of three sieves (2000 µm, 250 µm and 53 µm) was used to obtain the four aggregate-size classes: > 2000 µm (large macroaggregates (LM)), 250–2000 µm (small macroaggregates (SM)), 53–250 µm (microaggregates (MIC)) and < 53 µm (silt and clay (SC)). The separation of water-stable aggregates was achieved by manually moving the sieve up and down with 50 repetitions during a 2 min period. Each aggregate-size class was transferred into aluminum pans and dried at 60 °C until a constant weight was reached.

2.4. Carbon analysis

All solid samples were ground with a ball mill (Retsch, type MM). 15–20 mg of bulk soil and of isolated soil aggregates were placed into tin capsules to determine stable carbon ($\delta^{13}C$) isotope composition, as well as C and N contents. The same procedure was applied with two milligrams of roots for each depth on composite samples. Stable carbon

($\delta^{13}C$) isotope composition was determined for bulk soil for each soil depth (down to 45 cm). For soil aggregates no $\delta^{13}C$- values were determined for 30–45 cm soil depth in November 2011. Consequently, C-content and MRT of aggregates are shown down to depths of 30 cm, while of bulk soil down to 45 cm. Samples collected between 1997 and December 2005 were measured using a continuous flow, isotope ratio mass spectrometer (CF-IRMS, PDZ-Europa Scientific, Sandbach UK) interfaced with a CN analyzer (Carlo Erba). Samples collected from June 2006 till June 2007 were measured on a combined elemental analyzer and gas purification module (SerCon-GSL). Samples from November 2011 were analyzed on an isotope mass spectrometer (IRMS, DeltaXP Plus, Thermo Finnigan, Waltham, USA) and for December 2015 on a IRMS (GV Isoprime combined with an Elemental analyzer, Eurovector EA).

2.5. Estimation of C saturation and C saturation deficit

We determined C saturation (C_{sat}) of our study site for different soil depths by applying a model where C_{sat} is related to the silt and clay content in grassland (Six et al., 2002) (1).

$$C_{sat} = 16.33 + 0.32\,(Clay + Silt) \quad (1)$$

where C_{sat} is the C saturation (g C kg^{-1} soil) expressed as the C content of the Clay + Silt fraction on a whole-soil basis and Clay + Silt is the clay and silt (0–50 μm particles) contents (%). We used the soil texture data as presented in Table 1 and allocated the soil horizons to the increments of soil sampling. We did not present any results of C_{sat} for the depth 7.5–15 cm since we could not allocate a specific soil horizon to this depth (Table 1).

We then estimated C saturation deficit (C_{def}) according to Angers et al. (2011) (2), where the deficit is determined by the difference between the theoretical saturation and the actual stable SOC (SSOC) content.

$$C_{def} = C_{sat} - SSOC \quad (2)$$

where SSOC is stable SOC which is bound to minerals. SSOC was estimated to account for 78.63 ± 6.15% of SOC content in 0–7.5 cm, 94.15 ± 2.21% in 7.5–15 cm, 95.74 ± 1.77% in 15–30 cm and 96.38 ± 1.78% in 30–45 cm soil depth. According to Schrumpf et al. (2013) we determined the contribution of the free light fraction to the SOC content for different soil depths of three grassland sites. We applied these values as estimates of the unbound part of SOC to our grassland study site. Our estimate of 21.37 ± 6.15% for the fraction of SSOC in topsoil is in agreement with an average value of 20.8 ± 10.9% for the unbound part of SOC from 22 grassland sites (review by Gregorich et al. (2006)).

2.6. Assessment of aggregate-SOC content

We reported aggregate-SOC content in two ways. Mostly, we presented aggregate-SOC content on a whole soil basis (g C kg^{-1} soil) as this unit integrates the C concentration of the aggregate-size class (g C kg^{-1} aggregate) as well as the distribution of aggregate-size classes (g aggregate kg^{-1} soil). Additionally, we presented aggregate-SOC content in the unit g C kg^{-1} aggregate to elucidate if eCO_2 caused a change in the proportion of SOC within a given soil aggregate-size class (internal aggregate-SOC content).

2.7. Calculation of C input (C_{new}) and mean residence times (MRT)

The depleted $\delta^{13}C$ signature in the eCO_2 treatments enabled the application of an isotope mixing model to calculate the proportions of C_{new} that has been fixed since the change in $\delta^{13}C$ signature in July 2004 according to Equation (3) (Balesdent and Mariotti, 1996):

$$fC_{new} = \frac{\delta(t_1) - \delta(t_0)}{\delta_B - \delta(t_0)} \quad (3)$$

where fC_{new} is the fraction of new C in the SOC pool, $\delta(t_1)$ is the $\delta^{13}C$ signature of SOC in the elevated plots at t_1, $\delta(t_0)$ is the $\delta^{13}C$ signature of SOC in the elevated plots at t_0 and δ_B is the corresponding $\delta^{13}C$ signature of root biomass at t_1. We chose the $\delta^{13}C$ of root material because root material is the main input at the grassland study site as above ground biomass is harvested from the study plots (mimicking silage production).

Equation (3) was applied for soil aggregate-size classes and bulk soil at different soil depths. To calculate the absolute C_{new} content (g C_{new} kg^{-1} soil) we multiplied the relative fraction of C_{new} (g C_{new} $100\,g^{-1}$ SOC), which we derived from equation (3) with the SOC content of the corresponding aggregate-size class.

MRT of SOC in soil aggregate-size classes in different soil depths were estimated based on changes in their $\delta^{13}C$ over time after the switch in the signature of $^{13}CO_2$ in 2004. MRT of C in a pool (bulk soil or soil aggregate-size class) was defined as the average time required to completely renew the content of C in the pool at steady state (Six and Jastrow, 2002).

To describe changes in $\delta^{13}C$ vs. time, non-linear regressions of the form of $C_t = C_0 \cdot e^{-kt}$ were fitted to the data using SigmaPlot (ver 12.5, Systat Software Inc.). The equation was fitted to the C_{old} data vs. time, where $C_{old} = 1 - C_{new}$. C_{old} was forced to be equal to 1.0 at time zero (June 2004). The coefficient k is the first order decay constant for the organic matter pool and was derived from fitting the model to the data. C_t is the amount of C_{old} at the respective time t, t is the elapsed time since the signature switch of $\delta^{13}C$ in July 2004 and C_0 is the initial C content before the switch of the ^{13}C signature. MRT was then calculated as: $MRT = \frac{1}{k}[years]$. For estimation of MRT we included the earliest data from June 2007, as from this date on the ^{13}C signature was significantly different between aCO_2 and eCO_2 in all aggregate-size classes in the top 30 cm depth. Lower soil depths did not show sufficient change in their ^{13}C signature at this time and therefore no MRT could be estimated.

2.8. Data analysis

A General Linear Model (SPSS, version 24) was used to calculate univariate analysis of variance (ANOVA) and to evaluate CO_2 effects on soil aggregate-size classes in 0–7.5 cm depth at the full time series (1998–2015) and for the soil profile data which incorporated measurements from 6, 9 and 13.5 years of the experiment. No transformation of data was required as results of a Shapiro-Wilk-Test verified normal distribution of residuals. We split the data by aggregate-size class and by depth and applied separate ANOVAs to evaluate CO_2 effects in different depths and within soil aggregate-size classes. According to the experimental design the ANOVA model included the factors CO_2, block and time and their interactions.

To identify significant differences of MRT among aggregate-size classes we split the MRT data by depth and applied an ANOVA with the factor aggregate-size class. Significant differences of MRT within aggregate-size classes and between depths were performed by splitting the data by aggregate-size class and performing an ANOVA with the factor depth. Tukey's HSD was used as a post-hoc test to determine significant differences between groups. All effects and comparisons were considered significant at $p \leq 0.05$ and marginally significant at a p-value between 0.05 and 0.10.

3. Results

3.1. Distribution of aggregate-size classes in 0–7.5 cm depth within 17 years of eCO_2

Within the top 7.5 cm soil depth, a single observation showed an

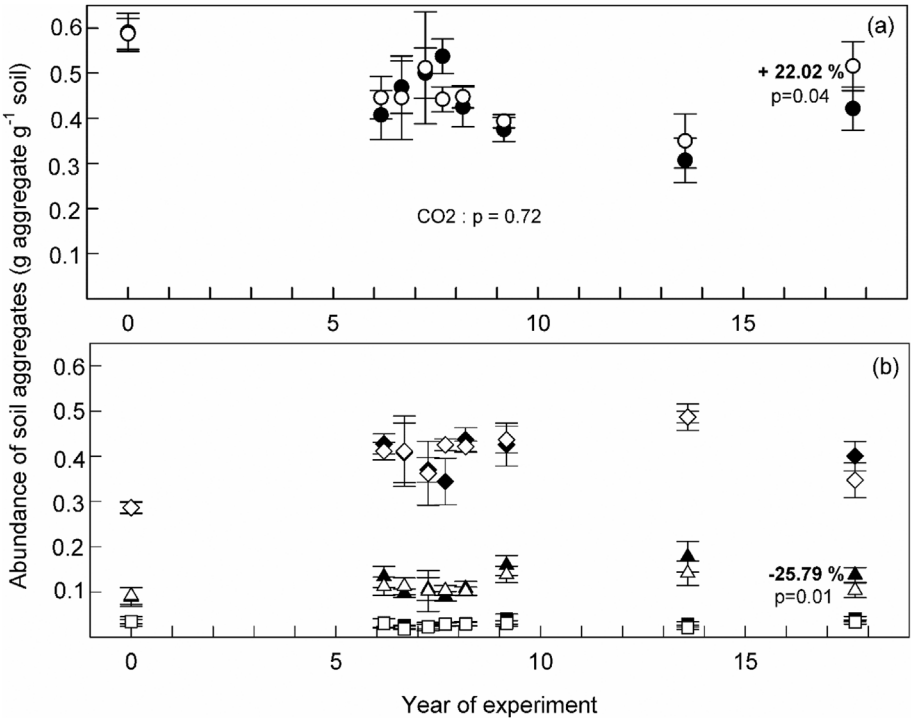

Fig. 1. Distribution of soil aggregate-size classes under aCO_2 (solid symbols) and eCO_2 (open symbols) in 0–7.5 cm soil depth during 17 years at the Gi-FACE experiment. Abundance of large macroaggregates (circles) **(a)**, small macroaggregates (diamonds), microaggregates (triangles) and silt and clay aggregates (squares) under aCO_2 (solid symbols) and eCO_2 (open symbols) in 0–7.5 cm soil depth **(b)**. Values are presented as means ± standard error, n = 3. Reported P values are for CO_2 effects.

Table 2
ANOVA table of effects of eCO_2 (CO_2), time and their interactions on the abundance of soil aggregate-size classes at the full time series (17 years of eCO_2) in 0–7.5 cm depth. Significant values are bolded.

Source	df	LM	SM	MIC	SC
		P	*P*	*P*	*P*
CO_2	1	0.724	0.525	**0.042**	**0.050**
Time	8	**0.000**	**0.000**	**0.000**	**0.001**
CO_2 x Time	8	0.519	0.449	0.450	0.742

LM: large macroaggregates, SM: small macroaggregates, MIC. microaggregates, SC: silt and clay.

eCO_2-induced increase in the abundance of LM by 22.02 ± 3.59% (p = 0.04) relative to aCO_2 after 17 years (Fig. 1a). However, this single observation of increased macroaggregation under eCO_2 did not impose a significant CO_2 effect on the whole investigation period in topsoil (Table 2). Increased macroaggregation after 17 years of eCO_2 was concomitant with a decreased abundance of MIC by 25.79% (p = 0.01) relative to MIC in aCO_2 plots (Fig. 1b).

Over the whole investigation period eCO_2 had no effect on the fraction of SM (p = 0.525) but decreased the fraction of MIC (p = 0.042) and SC (p = 0.050) in the top 7.5 cm soil depth (Table 2, Fig. 1b).

3.2. Soil aggregation effects in the soil profile within 13 years of eCO_2

Within the soil profile (0–45 cm depth) we observed CO_2-induced differences in soil aggregate-size distribution among depths (Table 3). While the abundance of LM increased in subsoil (15–45 cm depth) with a concomitant decrease in the abundance of SM (Fig. 2c + d), eCO_2 did not change the abundance of LM and SM in topsoil (0–15 cm depth) (Table 3, Fig. 2a + b). However, eCO_2 decreased the abundance of MIC and SC within the top 7.5 cm and in 15–45 cm soil depth (Table 3a – d).

Table 3a
Mass balance of aggregate-size classes and of aggregate-SOC content under aCO_2 and eCO_2 after 6, 9 and 13.5 years of the FACE experiment in 0-7.5 cm soil depth. Values are presented as means, n = 3 and reported P values show significant CO_2 effects. Significant values are bolded.

			0-7.5 cm depth			
Property	Year of experiment	Aggregate-size class	aCO2	eCO2	*df*	*P*
C content (g C kg-1 soil)	6	LM	15.74	17.47		
		SM	17.33	16.54		
		MIC	5.15	3.99		
		SC	0.81	0.96		
		total	**39.03**	**38.95**		
	9	LM	15.58	16.81		
		SM	18.52	19.64		
		MIC	5.41	5.07		
		SC	1.07	0.83		
		total	40.59	42.35		
	13.5	LM	11.69	12.91	1	0.270
		SM	14.78	15.29	1	0.773
		MIC	4.40	3.33	1	**0.079**
		SC	0.45	0.35	1	0.635
		total	**31.32**	**31.87**		
Abundance (g aggregate g-1 soil)	6	LM	0.41	0.45		
		SM	0.43	0.41		
		MIC	0.13	0.11		
		SC	0.03	0.03		
		total	**1.00**	**1.00**		
	9	LM	0.38	0.39		
		SM	0.43	0.44		
		MIC	0.16	0.14		
		SC	0.04	0.03		
		total	1.00	1.00		
	13.5	LM	0.31	0.35	1	0.165
		SM	0.49	0.49	1	0.937
		MIC	0.18	0.14	1	**0.035**
		SC	0.03	0.02	1	**0.087**
		total	**1.00**	**1.00**		

LM: large macroaggregates, SM: small macroaggregates, MIC: microaggregates, SC: silt and clay.

Table 3b
Mass balance of aggregate-size classes and of aggregate-SOC content under aCO_2 and eCO_2 after 6, 9 and 13.5 years of the FACE experiment in 7.5 - 15 cm soil depth. Values are presented as means, n = 3 and reported P values show significant CO_2 effects. Significant values are bolded.

			7.5-15 cm depth			
Property	Year of experiment	Aggregate-size class	aCO_2	eCO_2	*df*	*P*
C content (g C kg^{-1} soil)	6	LM	17.47	17.51		
		SM	12.02	10.36		
		MIC	2.98	1.82		
		SC	0.73	0.75		
		total	33.20	30.44		
	9	LM	15.40	20.40		
		SM	12.64	12.07		
		MIC	3.03	2.77		
		SC	0.56	0.48		
		total	31.63	35.73		
	13.5	LM	10.90	15.16	1	0.109
		SM	7.29	6.97	1	0.438
		MIC	3.66	2.08	1	**0.022**
		SC	0.47	0.63	1	0.748
		total	22.32	24.83		
Abundance (g aggregate g^{-1} soil)	6	LM	0.48	0.56		
		SM	0.36	0.33		
		MIC	0.12	0.08		
		SC	0.03	0.03		
		total	1.00	1.00		
	9	LM	0.46	0.52		
		SM	0.38	0.35		
		MIC	0.13	0.11		
		SC	0.03	0.02		
		total	1.00	1.00		
	13.5	LM	0.43	0.53	1	0.167
		SM	0.36	0.31	1	0.260
		MIC	0.18	0.13	1	0.111
		SC	0.03	0.03	1	0.172
		total	1.00	1.00		

LM: large macroaggregates, SM: small macroaggregates, MIC: microaggregates, SC: silt and clay.

Table 3c
Mass balance of aggregate-size classes and of aggregate-SOC content under aCO_2 and eCO_2 after 6, 9 and 13.5 years of the FACE experiment in 15-30 cm soil depth. Values are presented as means, n = 3 and reported P values show significant CO_2 effects. Significant values are bolded.

			15-30 cm depth			
Property	Year of experiment	Aggregate-size class	aCO_2	eCO_2	*df*	*P*
C content (g C kg^{-1} soil)	6	LM	8.39	10.72		
		SM	4.02	2.36		
		MIC	0.97	0.63		
		SC	0.41	0.26		
		total	13.79	13.98		
	9	LM	7.07	10.29		
		SM	6.91	5.58		
		MIC	1.76	1.16		
		SC	0.49	0.41		
		total	16.23	17.45		
	13.5	LM	9.32	12.89	1	**0.015**
		SM	5.81	5.18	1	0.100
		MIC	2.62	1.52	1	**0.005**
		SC	0.48	0.40	1	**0.016**
		total	18.23	19.99		
Abundance (g aggregate g^{-1} soil)	6	LM	0.54	0.76		
		SM	0.30	0.16		
		MIC	0.12	0.06		
		SC	0.04	0.02		
		total	1.00	1.00		
	9	LM	0.41	0.58		
		SM	0.40	0.31		
		MIC	0.15	0.09		
		SC	0.04	0.03		
		total	1.00	1.00		
	13.5	LM	0.40	0.55	1	**0.000**
		SM	0.38	0.30	1	**0.002**
		MIC	0.19	0.12	1	**0.000**
		SC	0.04	0.03	1	**0.005**
		total	1.00	1.00		

LM: large macroaggregates, SM: small macroaggregates, MIC: microaggregates, SC: silt and clay.

3.3. Aggregate-SOC content on a whole soil basis (g C kg^{-1} soil)

Elevated CO_2 increased the SOC content of LM in 15–30 cm soil depth (p = 0.015) (Table 3c, Fig. 3c) but not in the top 15 cm of soil (Table 3a + b, Fig. 3a + b) and significantly decreased the SOC content of MIC in all soil depths (Table 3a–c, Fig. 3a–c), while SOC in SC was decreased in 15–30 cm soil depth (Table 3a – c, Fig. 3a–c).

3.4. Internal aggregate-SOC content (g C kg^{-1} aggregate)

Internal aggregate-SOC content increased in SC in 7.5–30 cm but not in the top 7.5 cm soil depth under eCO_2 (Table 4). Internal SM-SOC increased under eCO_2 in 7.5–15 cm depth (Table 4). No change in internal LM-SOC was observed under eCO_2 (Table 4).

3.5. SOC content of bulk soil in the soil profile

Over the whole investigation period no change in SOC content of bulk soil was observed in any soil depth (Table 5, Fig. 4).

3.6. SOC saturation and saturation deficit in the soil profile

Our estimates of C_{sat} were similar for top- and subsoil, while SSOC and C_{def} differed among soil depths (Table 6). SSOC decreased with soil depth. In the top 7.5 cm of soil C_{def} was close to C_{sat} with a mean value of 4.07 ± 3.16 g C kg^{-1} soil for all plots. In subsoil C_{def} was 24.20 ± 1.99 g C kg^{-1} soil in 15–30 cm and 31.22 ± 3.71 g C kg^{-1}

Table 3d
Mass balance of aggregate-size classes and of aggregate-SOC content under aCO_2 and eCO_2 after 6, 9 and 13.5 years of the FACE experiment in 30-45 cm soil depth. Values are presented as means, n = 3 and reported P values show significant CO_2 effects. Significant values are bolded.

			30-45 cm depth			
Property	Year of experiment	Aggregate-size class	aCO_2	eCO_2	*df*	*P*
Abundance (g aggregate g^{-1} soil)	6	LM	0.22	0.34		
		SM	0.47	0.44		
		MIC	0.23	0.17		
		SC	0.08	0.05		
		total	1.00	1.00		
	9	LM	0.22	0.30		
		SM	0.43	0.41		
		MIC	0.27	0.22		
		SC	0.08	0.07		
		total	1.00	1.00		
	13.5	LM	0.22	0.38	1	**0.003**
		SM	0.43	0.38	1	**0.080**
		MIC	0.29	0.20	1	**0.005**
		SC	0.06	0.04	1	**0.059**
		total	1.00	1.00		

LM: large macroaggregates, SM: small macroaggregates, MIC: microaggregates, SC: silt and clay; C content is not presented in 30-45 cm since no $\delta^{13}C$- values were determined at this soil depth.

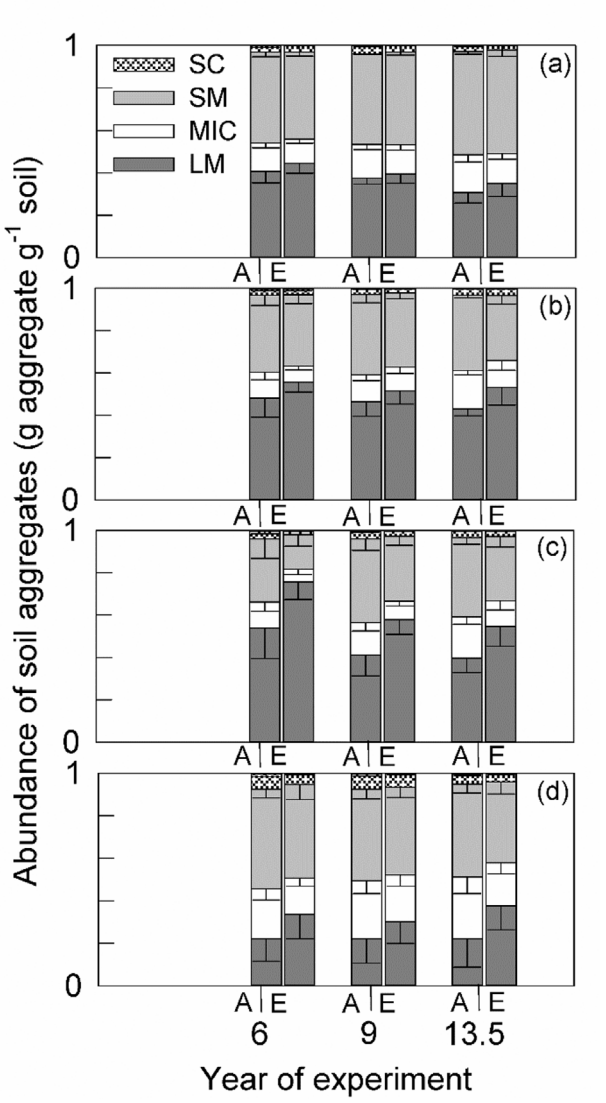

Fig. 2. Distribution of aggregate-size classes under aCO_2 (A) and eCO_2 (E) in 0–7.5 cm (a), 7.5–15 cm (b), 15–30 cm (c) and 30–45 cm (d) soil depth. Values are presented as means ± standard error, n = 3. LM: large macroaggregates, SM: small macroaggregates, MIC:microaggregates, SC: silt and clay aggregates.

soil in 30–45 cm (Table 6).

3.7. Soil C input in the CO_2 enriched plots and MRT

Highest absolute amounts of C_{new} (g C_{new} kg^{-1} soil) were found in SOC of bulk soil in the top 7.5 cm of soil (Table 7). C_{new} of bulk soil was significantly higher than in any soil aggregate-size class at this soil depth (Table 7). Among soil aggregate-size classes absolute amounts of C_{new} differed between macroaggregates and SC and between SM and MIC (Table 7). In lower soil depths bulk soil and macroaggregates showed the highest absolute amounts of C_{new} (Table 7). C_{new} in bulk soil was significantly higher than in MIC and SC in 7.5–30 cm soil depth (Table 7). C_{new} in SM, MIC and bulk soil was significantly lower in 7.5–15 and 15–30 cm soil depth than in the top 7.5 cm soil depth, while LM and SC did not differ in their C_{new} content among soil depths (Table 7).

MRT of SOC in soil aggregate-size classes were not different among aggregate-size classes at the same depth (Table 7). However, MRT of SOC in macroaggregates and bulk soil was significantly different among top- and subsoil (Table 7). We did not observe any significant differences of the MRT among depths for MIC and SC (Table 7).

4. Discussion

4.1. Changes in SOC content and distribution of aggregate-size classes

In contrast to our initial hypotheses, long-term eCO_2 did not change the SOC content of bulk soil in any depth (Fig. 4, Table 5). Despite our estimations of high SOC sequestration potential (C_{def}) in subsoil of the grassland ecosystem (Table 6), we did not observe an increased SOC content in subsoil within 13 years of eCO_2. In topsoil, for which we estimated a small SOC sequestration potential (C_{def}), we also did not observe an increase in SOC content under eCO_2.

There have been recent discussions on the suitability of the applied $C_{sat\text{-}def}$ concept for assessing the bulk soil SOC sequestration potential (Barré et al., 2017). It was criticized that C_{sat} based on the fine fraction does not account for C of coarse fractions such as particulate organic matter or sand-sized particles. We are aware that these aspects may limit the accuracy of the estimated C_{sat} and following C_{def} values. However, we took account of the fraction of unbound POM-C and incorporated the SOC bound to minerals (SSOC) into our equation (2). Despite the known limitations, our results of higher C_{def} in sub- than topsoil would arguably also persist with more detailed modelling approaches as they are in line with other studies (Kaiser and Guggenberger, 2003).

Despite no changes in bulk SOC content between CO_2 treatments we found a depth-dependent response in macroaggregation. We observed CO_2 induced macroaggregation in 15–45 cm depth but not in topsoil. Consequently, increased macroaggregation in subsoil did not result in C sequestration at the study site.

Even though we did not detect an increased SOC content in subsoil we found that LM-SOC content increased concomitantly with a decreased SOC content in MIC and SC. Consequently, increased LM-SOC content on a whole soil basis may have been counterbalanced by decreases in MIC and SC fractions. The analysis of internal aggregate-SOC content provided a different picture of SOC dynamics: Despite CO_2 induced increases of LM-SOC on a whole soil basis we did not observe any difference in internal LM-SOC content between CO_2 treatments. This may also explain why we did not detect any increased SOC content in bulk soil under eCO_2.

SC actually increased in their internal SOC content in 7.5–30 cm soil depth under eCO_2. However, the observed decrease of the SC fraction probably outbalanced the increase in SOC content, as seen on a whole soil basis. The increase in internal SC-SOC content are in line with our findings that SC-SOC contained a high fraction of C_{new} in 7.5–30 cm soil depth relative to the other aggregate-size classes (Table 7). These findings support the concept that subsoils possess a higher fraction of unsaturated mineral surfaces than topsoil where organic substances can be absorbed to (Poirier et al., 2014). However, this could not be confirmed for other aggregate-size classes or bulk soil as we did not observe any concomitant increase in internal SOC content. Decreased abundance of SC fractions under eCO_2 may be explained by absorption of organic substances to these particles and subsequent formation of macroaggregates (Blanco-Canqui and Lal, 2004).

However, no changes in bulk SOC under eCO_2 are in line with observations from other FACE experiments (Table S1) (Six et al., 2001; van Groenigen et al., 2002; del Galdo et al., 2006; Lichter et al., 2008) but contrast observations by Hoosbeek et al. (2006) and Hofmockel et al. (2011).

As the SOC content at a given time represents the balance between C inputs und losses we argue that the increase of C_{new} under eCO_2 may be counterbalanced by the rate of microbial decomposition resulting in no net C increase in SOC. This is in accordance with earlier findings from the Gi-FACE reporting increased soil respiration rates under eCO_2 in late autumn and winter (Keidel et al., 2015).

Macroaggregation has been related to temporary binding agents

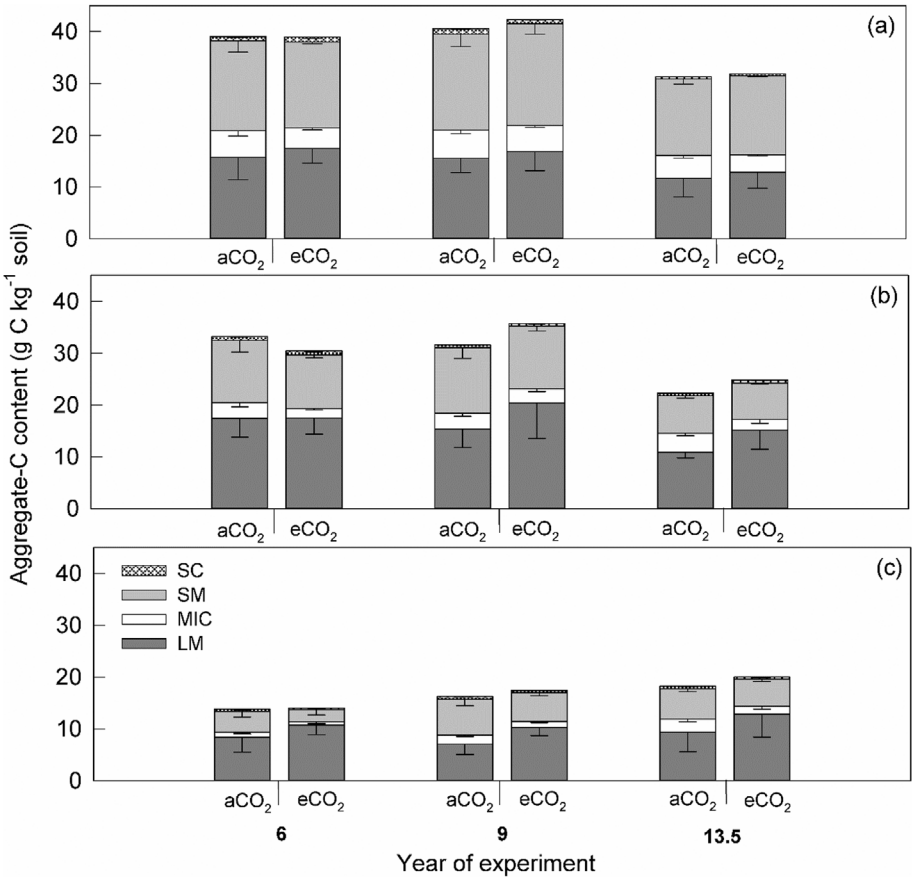

Fig. 3. Aggregate-C content under aCO_2 and eCO_2 in 0–7.5 cm (a), 7.5–15 cm (b), 15–30 cm (c) soil depth after six, nine and 13.5 years. Values are presented as means ± standard error, n = 3. C content is not presented in 30–45 cm since no $\delta^{13}C$- values were determined at this soil depth after 13.5 years. LM: large macroaggregates, SM: small macroaggregates, MIC:microaggregates, SC: silt and clay aggregates.

Table 4
ANOVA table of effects of eCO_2 on internal aggregate-SOC content (g C kg^{-1} aggregate) after six, nine and 13.5 years of CO_2 enrichment in different soil depths. Significant values are bolded.

Depth	*df*	LM	SM	MIC	SC
		P	*P*	*P*	*P*
0–7.5 cm	1	0.723	0.544	0.938	0.155
7.5–15 cm	1	0.307	**0.051**	0.689	**0.041**
15–30 cm	1	0.802	0.452	0.175	**0.062**

LM: large macroaggregates, SM: small macroaggregates, MIC. microaggregates, SC: silt and clay. No $\delta^{13}C$- data was available for soil aggregate size classes in 30–45 cm soil depth after 13.5 years.

Table 5
ANOVA table of effects of eCO_2 on SOC content of bulk soil at different soil depths.

Depth	*df*	bulk soil
		P
0–7.5 cm	1	0.866
7.5–15 cm	1	0.367
15–30 cm	1	0.471
30–45 cm	1	0.129

such as roots and fungal hyphae (Tisdall and Oades, 1982). However, more recent studies reported that higher root length densities increased the proportions of smaller aggregates (Materechera et al., 1992). Increased root biomass was often observed in response to eCO_2 (Jastrow et al., 2000; Eviner and Chapin, 2002), however, at Gi-FACE there is no such evidence because even after 13 years of eCO_2 no CO_2 effect on root biomass was observed over the soil profile (0–45 cm depth) (Fig. S2).

Still, fungal-derived binding agents cannot be ruled out to be responsible for the observed increase in macroaggregation (Rillig et al., 1999). Glomalin has been linked to aggregate stability (Wright and Upadhyaya, 1998). Rillig et al. (1999) reported an increased glomalin content and macroaggregate abundance under eCO_2, and concluded that arbuscular mycorrhizal fungi (AMF) mediated the CO_2-induced increase in soil aggregation. However, recent studies question that glomalin originates from AMF and refer to it as glomalin-related soil protein (Gillespie et al., 2011). In a different study, Rillig and Field, 2003 reported that AMF responses to plants exposed to eCO_2 followed a soil-depth dependent pattern. About 5-fold increases of AM fungal root colonization were observed in the subsoil in response to eCO_2, but no significant changes in the corresponding topsoil of *Bromus hordeaceus* L. This is in line with observations from a forest FACE study, where CO_2 enrichment increased mycorrhizal root tip production in deep soil (15–30 cm) but did not influence mycorrhizal production in shallow soil (0–15 cm) (Pritchard et al., 2008).

To date studies of AMF at the Gi-FACE were limited to the topsoil layer showing no CO_2 induced increases in abundance of AMF (Gerstner, 2014) after 15 years of eCO_2. Our results point out that studies on AMF should also include subsoil layers in CO_2 enrichment experiments to test if a CO_2-induced increase in AMF colonization can explain increases in soil aggregation in the subsoil.

4.2. Soil C input in the CO_2 enriched plots and MRT

We suggest that highest amounts of C_{new} in bulk soil in the top 7.5 cm of soil may be explained by a relative high fraction of C_{new} in free particulate organic matter (POM) that was not occluded within soil aggregates at this soil depth.

The relative high fraction of C_{new} in SC may partly result from wet

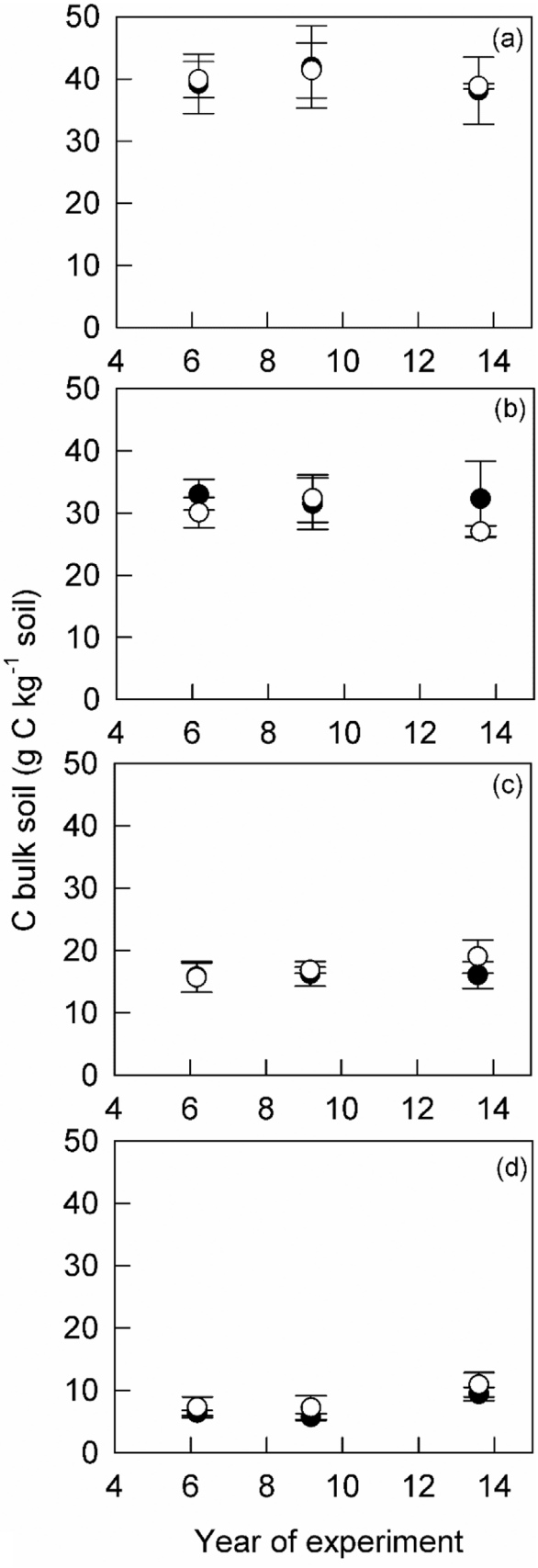

Fig. 4. SOC content of bulk soil under aCO_2 (solid circles) and eCO_2 (open circles) in 0–7.5 cm (a), 7.5–15 cm (b), 15–30 cm (c) and 30–45 cm (d) soil depth after six, nine and 13.5 years. Values are presented as means ± standard error, n = 3.

Table 6
C saturation (C_{sat}), stable soil organic carbon content (SSOC) and C saturation deficit (C_{def}) estimated for the grassland study site at different soil depths after 6 years of the FACE experiment. Values are presented as means of all rings, based on ring pairs (n = 3). Different letters represent significant differences among soil depths ($p > 0.1$).

Soil depth	C_{sat}		SSOC		C_{def}	
	(g C kg^{-1} soil)					
0–7.5 cm	36.33 a	± 4.64	32.26 a	± 4.05	4.07 a	± 3.16
15–30 cm	39.24 a	± 3.04	15.04 b	± 1.17	24.20 b	± 1.99
30–45 cm	37.84 a	± 3.09	6.61 c	± 0.95	31.22 c	± 3.71

Soil depth 7.5–15 cm is not presented since it could not be assigned to a particular soil horizon (Table 1).

sieving where soluble C associated with micro- and macroaggregates may have entered the SC fraction which are known to absorb organic substances to its surfaces (Blanco-Canqui and Lal, 2004). However, due to the small pool size of this aggregate-size class, high relative values had only a negligible influence on the absolute amount of C_{new} (Table 7). The high fraction of C_{new} in SC resulted in relatively fast MRT of SOC within this aggregate-size class (Table 7).

Our study showed that the MRT of SOC in different aggregate-size classes did not differ significantly among each other. However, macroaggregates and bulk soil differed in their MRT between soil depths. These results are in contrast to other experiments where MRT of SOC increased with aggregate size (Six et al., 2001). Our observations are also in contrast to results from a review of Von Lützow et al. (2007) reporting MRT of about 15–50 years for SOC in macroaggregates and 100–300 years for SOC in microaggregates. On the other hand, van Groenigen et al. (2002) found no significant differences in C_{new} between aggregate-size classes under eCO_2 and suggested that this was due to the high level of aggregation and the incorporation of MIC into macroaggregates. In line with these results we suggest that similar values of C_{new} in subsoil and consequently similar MRT at the Gi-FACE study may be caused by aggregation dynamics under eCO_2.

5. Conclusions

The study of 17 years of moderate CO_2 enrichment showed that despite an estimated high SOC sequestration potential of the grassland subsoil and an increased macroaggregation under eCO_2 no increase in total SOC content under eCO_2 could be observed. However, we found a CO_2 induced increase in LM-SOC on a whole soil basis but no internal LM-SOC increase in subsoil. SC aggregates also showed a depth-dependent pattern with internal SOC increases in lower soil depths. Since the MRT of macroaggregates and the bulk soil was higher in subsoil than in topsoil, C_{new} allocated to these depths at the grassland study site will be sequestered for longer periods than in topsoil. We conclude from our study that approaches estimating the SOC sequestration potential, based on the fraction of silt and clay particles, may not reflect appropriately the actual SOC sequestration under eCO_2. The investigation of soil aggregates provided insight into the C protection dynamics and C allocation patterns under eCO_2.

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Table 7
Relative and absolute amounts of C_{new}, k-value and MRT of SOC in soil aggregate-size classes and bulk soil after 13.5 years of eCO_2. Values are presented as means ± standard error, n = 3. Results of a Tukey's HSD post-hoc test show significant differences among aggregate-size classes and among soil depths for C_{new}. Different uppercase letters indicate significant differences among aggregate-size classes within same depth for MRT. Different lowercase letters indicate significant differences of aggregate-size classes among depths for MRT.

Depth	aggregate-size class	Cnew			Tukey's HSD comparisons					k			MRT	
(cm)		(g 100 g^{-1} SOC)		(g kg^{-1} soil)	LM	SM	MIC	SC	bulk soil	0–7.5	7.5–15	15–30	(yr)	
0–7.5	LM	24.42	± 0.01	3.07 ± 0.06				0.044	< 0.01				0.038 ± 0.00	27 ± 2.05 Aa
	SM	26.44	± 0.02	4.04 ± 0.03			0.022	< 0.01	< 0.01		< 0.01	< 0.01	0.041 ± 0.00	25 ± 2.08 Aa
	MIC	19.17	± 0.01	0.63 ± 0.15		0.022			< 0.01		0.043	0.041	0.029 ± 0.01	41 ± 9.70 Aa
	SC	20.09	± 0.03	0.07 ± 0.01	0.044	< 0.01			< 0.01				0.030 ± 0.01	35 ± 4.70 Aa
	Bulk soil	30.57	± 0.03	11.85 ± 1.25	< 0.01	< 0.01	< 0.01	< 0.01			0.007	0.002	0.049 ± 0.01	21 ± 2.90 Aa
7.5–15	LM	16.99	± 0.02	2.73 ± 1.02									0.025 ± 0.00	42 ± 5.62 Aa
	SM	17.65	± 0.02	1.23 ± 0.15						< 0.01			0.026 ± 0.00	39 ± 3.59 Ab
	MIC	9.51	± 0.02	0.18 ± 0.06						0.043			0.013 ± 0.00	81 ± 15.66 Aa
	SC	19.30	± 0.05	0.13 ± 0.06									0.029 ± 0.01	40 ± 9.23 Aa
	Bulk soil	14.56	± 0.05	4.03 ± 1.50			0.042	0.040		0.007			0.021 ± 0.01	68 ± 29.28 Aa
15–30	LM	15.26	± 0.02	2.13 ± 1.02				0.084					0.022 ± 0.00	47 ± 7.23 Ab
	SM	11.50	± 0.01	0.60 ± 0.02						< 0.01			0.016 ± 0.00	62 ± 4.93 Ac
	MIC	11.66	± 0.04	0.18 ± 0.02					0.094	0.041			0.017 ± 0.01	79 ± 30.88 Aa
	SC	18.10	± 0.04	0.07 ± 0.02	0.084				0.074				0.027 ± 0.01	41 ± 9.21 Aa
	Bulk soil	10.35	± 0.02	2.18 ± 0.41			0.094	0.074		0.002			0.015 ± 0.00	76 ± 19.00 Ab

LM: large macroaggregates, SM: small macroaggregates, MIC: microaggregates, SC: silt and clay. No $\delta^{13}C$- data was available for soil aggregate size classes in 30–45 cm soil depth after 13.5 years.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2018.05.005.

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